

## Original article:

# ANTIOXIDANT AND ANTIULCER POTENTIAL OF AQUEOUS LEAF EXTRACT OF *KIGELIA AFRICANA* AGAINST ETHANOL-INDUCED ULCER IN RATS

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## ABSTRACT

Ethnobotanical claims regarding *Kigelia africana* reported antiulcer properties as part of its medicinal application. In this work, aqueous leaf extract from *K. africana* was investigated for its phytochemical constituents and antiulcer potential against ethanol-induced ulcer in rats. The participation of oxidative stress on ethanol-induced ulcer and the potential protective antioxidant activity of *K. africana* extracts were investigated by determining vitamin C and thiobarbituric acid reactive species (TBARS) contents in the gastric mucosa of rats. The HPLC analysis showed the presence of gallic acid, chlorogenic acid, caffeic acid and also the flavonoids rutin, quercetin and kaempferol in the aqueous plant extract. Oral treatment with *K. africana* extract (1.75; 3.5; 7 and 14 mg/kg) one hour after ulcer induction with ethanol decreased in a dose dependent manner the ulcer index. Ethanol increased significantly stomachal TBARS levels and decreased vitamin C content when compared to the control animals. *K. africana* blunted the ethanol-induced oxidative stress and restored vitamin C content to the control levels. The present results indicate that the aqueous leaf extract from *K. africana* possesses antiulcer potential. The presence of flavonoids in plant extract suggests that its antiulcerogenic potential is associated with antioxidant activity. Of particular therapeutic potential, *K. africana* was effective against ethanol even after the induction of ulcer, indicating that it can have protective and curative effects against gastric lesion.

**Keywords:** *Kigelia africana*, gastric damage, antioxidant activity, flavonoids, ethanol, antiulcerogenic potential

## INTRODUCTION

The use of traditional medicine is expanding globally. It continues to be used not only for primary health care in develo-

ping countries, but also in countries where conventional medicine is predominant in the national health care system (WHO, 2000). In South Africa, 60-80 % of the

population relies solely or partially on traditional herbal medicines to treat a variety of animal and human diseases (Dausdardt, 1990). In the late 1980s, an estimated 65 % of the world's population depended on or at least used medicines derived from plants in health care (Farnsworth, 1988).

*Kigelia africana* (Sausage tree) belongs to the family *Bignoniaceae* and in the South-western part of Nigeria hot-infusion preparations of its leaves are popularly used to treat stomach ulcer. This plant is widely spread across Africa and is abundantly found in wet savannah where it occurs in abundance. Traditional remedies prepared from crushed dried fruits are used for emollient, antieczema, antipsoriasis, as dressing for ulcers and wounds, treatment of skin cancer, as anaphrodisiac and also as an active ingredient in skin lightening and breast firming formulations (Maisiri and Gundidza, 1999). The polar extract of *K. africana* fruit contains verminoside as the major constituent and a series of other phenols (Verbascoside, Caffeic Acid, *p*-Coumaric acid and Caffeic acid methyl ester) (Picerno et al., 2005). Remedies from root bark are also used for the treatment of venereal diseases, hemorrhoids, and rheumatism (Oliver-Bever, 1986). However, little is known about the pharmacological properties of its leaves.

Previously, preliminary data from our laboratories have indicated that the ethanolic leaf extract of *K. africana* has antiulcerogenic potential against aspirin-induced ulcer (Olaleye, 2005). However, the molecular mechanism involved in the protection, namely, the role of oxidative stress was not investigated. Furthermore, there is dearth of information as regards the antiulcerogenic potential of the leaf extract of this plant against ethanol-induced ulcer. Pharmacological and scientific validation of the ethnobotanical claims regarding the plant is essential to move towards the use of the plant as a drug. Since the literature reports suggest the ethnobotanical use of this plant in stomach disorders and skin

ulceration, it becomes important to study the antiulcer potential of the extract against ethanol-induced gastric ulcer.

Ethanol-induced gastric ulcers have been widely used for the evaluation of gastroprotective activity of different compounds (Al-Shabanah, 1997; Hwang et al., 2008; Ineu et al., 2008). Furthermore, oxygen radicals and lipid peroxidation are thought to be involved in the ethanol-induced gastric damage and antioxidant compounds can have gastroprotective properties (Ineu et al., 2008). Of particular pharmacological importance, *K. africana* has both *in vitro* and *in vivo* antioxidant properties (Olaleye and Rocha, 2007, 2008) and there is no work about participation of the antioxidant effect of this plant on its antiulcer properties. This work is therefore designed to evaluate the potential protective effect of aqueous leaf extract from *K. africana* against ethanol-induced ulcer and the possible protection role of the extracts against oxidative stress in ethanol-induced ulcer in rats.

## MATERIALS AND METHODS

### **Sample Collection and Preparation**

The plant sample *Kigelia africana* (Sausage tree) was collected from Ado in Ekiti State, Nigeria. Authentication was done at the Biology Department of the Federal University of Technology, Akure, Nigeria. The leaves of the plants were cleaned, air dried at room temperature for two weeks, ground to a powder and boiled in hot water for 3 hours, filtered and concentrated to dryness. The % yield for the extract was about 20 %.

### **Bioassay**

Adult Wistar albino rats (160-250 g) were collected from our own breeding colony. They were all clinically healthy and maintained in standard environmental conditions of temperature ( $24 \pm 2$  °C), 12 h dark/light cycle. They were fed a standard diet and water *ad libitum*. The animals were used according to the guidelines of the

Committee on Care and Use of Experimental Animal Resources, the Federal University of Santa Maria RS, Brazil. The gastric lesions were induced with ethanol according to the method described by Robert (1979). After a 36 h fasting, the animals received a single dose of 0.5 ml of ethanol or water (control group) orally by gavage. Then, after one hour animals received 1 ml of water (control and ethanol group) or 1 ml of one of the doses of aqueous leaf extract of *K. africana* by gavage, resulting in six groups of five animals each as follows:

- Group I: Control
- Group II: Ethanol group [treated with 0.5 ml ethanol (70 %) only]
- Group III: Induced with 0.5 ml ethanol (70 %) and treated with *Kigelia africana* at 1.75 mg/Kg
- Group IV: Induced with 0.5 ml ethanol (70 %) and treated with *Kigelia africana* at 3.5 mg/kg
- Group V: Induced with 0.5 ml ethanol (70 %) and treated with *Kigelia africana* 7 mg/Kg
- Group VI: Induced with 0.5 ml ethanol (70 %) and treated with *Kigelia africana* at 14 mg/kg

One hour after the administration of extracts, the animals were anaesthetized and euthanized. Stomach was removed and cut open along the greater curvature. The stomachs were rinsed under a slow stream of water and pinned flat on a cork board. The stomachs were examined macroscopically to assess the degree of ulceration. The ulcerative lesion index (UI) of each animal was calculated according to the method of Gamberini et al. (1991). The stomach linings were scraped weighed and homogenized in 0.1 M phosphate buffer in 1:10 wt/vol and centrifuged for 15 minutes at 2000 ×g. The homogenates were used to assay for TBARS and Vitamin C.

#### **Lipid peroxidation**

The extent of gastric lipid peroxidation was determined using the method described

by Ohkawa et al. (1979) as slightly modified by Puntel et al. (2007).

#### **Ascorbic acid determination**

Ascorbic acid determination was performed as previously described by Jacques-Silva et al. (2001). Stomach protein content was precipitated in 10 volumes of cold 4 % trichloroacetic acid solution. An aliquot of the sample in a final volume of 1 mL of the solution was incubated for 3 h at 38 °C, and subsequently 1 mL H<sub>2</sub>SO<sub>4</sub> 65 % (v/v) was added to the medium. The reaction product was determined using a color reagent containing 4.5 mg/mL dinitrophenyl hydrazine and CuSO<sub>4</sub> (0.075 mg/mL) at 520 nm (Spectrophotometer U-2001 Hitachi, Japan).

#### **HPLC analysis of *Kigelia africana* crude extract**

All chemical were of analytical grade. Methanol, acetic acid, gallic acid, chlorogenic acid and caffeic acid purchased from Merck (Darmstadt, Germany). Quercetin, rutin and kaempferol were acquired from Sigma Chemical Co. (St. Louis, MO, USA). High performance liquid chromatography (HPLC-DAD) was performed with the HPLC system (Shimadzu, Kyoto, Japan), Prominence Auto Sampler (SIL-20A), equipped with Shimadzu LC-20AT reciprocating pumps connected to the degasser DGU 20A5 with integrator CBM 20A, UV-VIS detector DAD (diode) SPD-M20A and Software LC solution 1.22 SP1.

Reverse phase chromatographic analyses were carried out under gradient conditions using C<sub>18</sub> column (4.6 mm x 250 mm) packed with 5 µm diameter particles; the mobile phase was water containing 2 % acetic acid (A) and methanol (B), and the composition gradient was: 5 % of B until 2 min and changed to obtain 25 %, 40 %, 50 %, 60 %, 70 % and 100 % B at 10, 20, 30, 40, 50 and 80 min, respectively, following the method described by Laghari et al. (2011) with slight modifications. The aqueous extract of the plant was dissolved in water and analyzed at a concentration of

4 mg/mL. The presence of six phenolic compounds was investigated, namely, gallic, chlorogenic and caffeic acids and the flavonoids quercetin, rutin and kaempferol. Identification of these compounds was performed by comparing their retention time and UV absorption spectrum with those of the commercial standards. The flow rate was 0.6 ml/min, injection volume 40  $\mu$ l and the wavelength were 254 nm for gallic acid, 325 nm for caffeic and chlorogenic acids, and 365 nm for quercetin, rutin and kaempferol. All the samples and mobile phase were filtered through 0.45  $\mu$ m membrane filter (Millipore) and then degassed by ultrasonic bath prior to use. Stock solutions of standards references were prepared in the HPLC mobile phase at a concentration range of 0.031-0.250 mg/ml for kaempferol, quercetin and rutin; and 0.006-0.250 mg/ml for gallic, caffeic and chlorogenic acids. The chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200 to 500 nm). Calibration curve

- for gallic acid:  
 $Y = 11707x + 1459.8$  ( $r = 0.9996$ );
- chlorogenic acid:  
 $Y = 15882x + 1326.3$  ( $r = 0.9993$ );
- rutin:  
 $Y = 12473x - 1075.7$  ( $r = 0.9999$ );
- quercetin:  
 $Y = 16135x - 1092.6$  ( $r = 0.9997$ ) and
- kaempferol:  $Y = 16923x - 1353.9$  ( $r = 0.9998$ ).

All chromatography operations were carried out at ambient temperature and in triplicate.

### Statistical analysis

Data were analyzed statistically by one-way ANOVA, followed by Duncan's multiple range tests when appropriate. The results were considered statistically significant at  $p < 0.05$ .

## RESULTS

### Ulcer index

Ethanol caused a statistically significant increase in the ulcer index when compared to the control group ( $p < 0.05$ ) and *K. africana* treatment caused a dose dependent decrease in this index (Table 1). In fact, *K. africana* extract was effective at the lowest dose tested (1.75 mg/kg) and it had a maximal effect at the dose of 7 mg/kg (Table 1).

### Vitamin C

Ethanol caused a significant decrease of about 20 % in stomach vitamin C content ( $p < 0.05$ ) when compared to the control group. *K. africana* leaf extract (1.75 to 7 mg/kg) restored the vitamin C content to control levels. The dose of 14 mg/kg of *K. africana* extract increased vitamin C levels but this effect was not statistically significant (Figure 1).

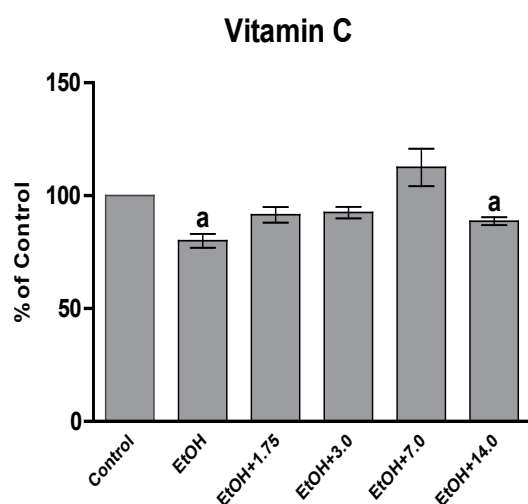
### TBARS

Ethanol caused statistically significant increase in the TBARS formation as compared to the control and treatment with *K. africana* abolished the pro-oxidant effect of ethanol (Figure 2).

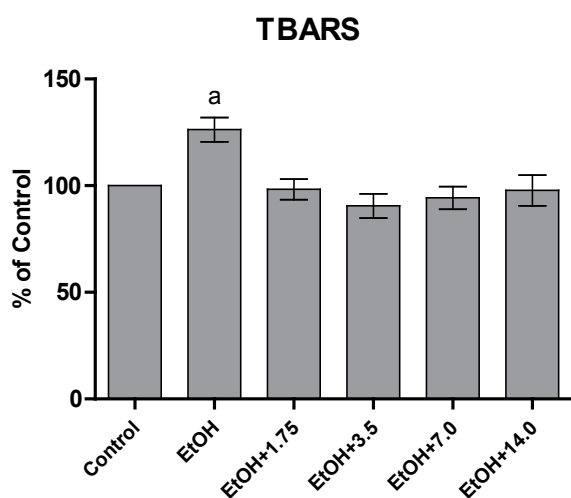
**Table 1:** Indexes of ethanol- induced stomachal ulcer in albino rats pos-treated with leaf extract of *Kigelia africana* as compared to the control

Groups	Ulcer index
Control	2.25 $\pm$ 0.39 <sup>a</sup>
Ethanol	16.25 $\pm$ 0.52 <sup>f</sup>
Ethanol + <i>Kigelia africana</i> (1.75 mg/Kg)	10.00 $\pm$ 0.34 <sup>e</sup>
Ethanol + <i>Kigelia africana</i> (3.50 mg/Kg)	6.50 $\pm$ 0.16 <sup>d</sup>
Ethanol + <i>Kigelia africana</i> (7.00 mg/Kg)	3.25 $\pm$ 0.21 <sup>b</sup>
Ethanol + <i>Kigelia africana</i> (14.00 mg/Kg)	4.75 $\pm$ 0.23 <sup>c</sup>

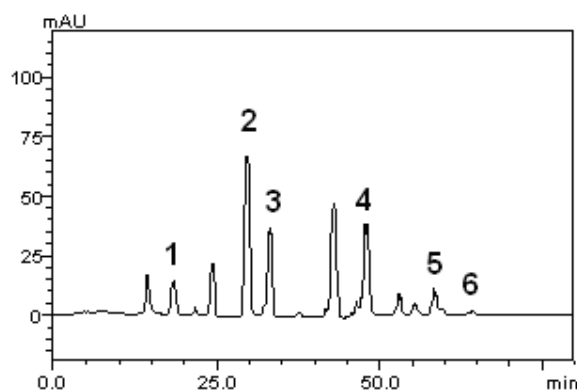
Values are expressed as means  $\pm$  SE of 5 replicates. Values carrying the same superscript down the group are not statistically significant different ( $p < 0.05$ ).



**Figure 1:** Vitamin C content of control, ethanol and *K. africana* treated rats. Values are expressed as means  $\pm$  SE of 5 replicates. The letter “a” indicates significant difference from control group ( $p < 0.05$ ). Columns marked with the same letter are not statistically different.



**Figure 2:** TBARS levels of control, ethanol and *K. africana* treated rats. Values are expressed as means  $\pm$  SE of 5 replicates. The letter “a” indicates significant difference from control group ( $p < 0.05$ ).



**Figure 3:** Representative high performance liquid chromatography profile of *K. africana*. Detection UV was at 325 nm. Gallic acid (peak 1), chlorogenic acid (peak 2), caffeic acid (peak 3), rutin (peak 4), quercetin (peak 5) and kaempferol (peak 6). Chromatographic conditions are described in the Methods section.

**Table 2:** Phenolics and flavonoids composition of aqueous extract of leaves from *K. africana*

Compounds	<i>K. africana</i>	
	mg/g	%
Gallic acid	11.93 $\pm$ 0.06 <sup>a</sup>	0.45
Chlorogenic acid	2.62 $\pm$ 0.04 <sup>b</sup>	1.70
Caffeic acid	8.35 $\pm$ 0.09 <sup>c</sup>	0.95
Rutin	25.30 $\pm$ 0.07 <sup>d</sup>	1.01
Quercetin	7.94 $\pm$ 0.15 <sup>e</sup>	0.32
Kaempferol	14.42 $\pm$ 0.11 <sup>f</sup>	0.08

Values are expressed as means  $\pm$  SE of 3 replicates. Values carrying the same superscript down the group are not statistically significant different ( $p < 0.01$ ).

### HPLC analysis

HPLC fingerprinting of *Kigelia africana* aqueous extracts revealed the presence of the

- gallic acid ( $t_R = 17.07$  min; peak 1),
- chlorogenic acid ( $t_R = 30.11$  min peak 2),
- caffeic acid ( $t_R = 34.27$  min peak 3),
- rutin ( $t_R = 47.94$  min; peak 4),
- quercetin ( $t_R = 55.83$  min; peak 5) and
- kaempferol ( $t_R = 64.56$  min; peak 6) (Figure 3 and Table 2).

## DISCUSSION

Plant extracts are among the most attractive sources for developing new drugs and have been shown to produce promising results in the treatment of gastric ulcer (Hiruma-Lima et al., 2000a, b, 2001). Accordingly, in traditional medicine several plants with antioxidant properties have been used to treat gastrointestinal disorders (Olaleye and Rocha, 2007, 2008; Sabir and Rocha, 2008; Sabir et al., 2012; Leite et al., 2009) including gastric ulcers (Toma et al., 2002). Plants continue to play a major role as therapeutic remedies in primary health care in developing countries and the use of aqueous extracts are very common for the population around the world (McDonald et al., 2001). Here, we have demonstrated that aqueous extract of *Kigelia africana* protected the gastric mucosa decreasing in a dose dependent manner the ulcer index caused by ethanol.

Of particular importance, literature data have indicated the antiulcer properties of flavonoids (Gonzalez and Di Stasi, 2002; Gracioso et al., 2002). The analyses of the plant extract by HPLC revealed the presence of several polyphenolic compounds such as Gallic acid, Chlorogenic acid, Caffeic acid, rutin, quercetin and kaempferol. The flavonoids rutin, quercetin and kaempferol, which were found in relatively high concentrations in aqueous extract of *K. Africana*, are secondary metabolites present in plants and have attracted the attention of many researchers because the wide range of their biological activities (Harborne, 1996; Middleton et al., 2000; Manthey et al., 2001; Zaveri, 2006)

Reactive oxygen species (ROS) can cause deleterious effects at the cellular level which can culminate in cell death. In line with this, the increase in gastric TBARS contents and the decrease in vitamin C levels after ethanol administration suggest that oxidative stress play an important role in ethanol-induced ulcer, which is in accordance with literature data (Ineu et al., 2008; Glavin and Szabo, 1992). The ability

of *K. africana* extract to ameliorate the gastric damage and to normalize vitamin C and TBARS contents indicate that its anti-ulcer effect was mediated, at least in part, by its antioxidant activity (Olaleye and Rocha, 2007, 2008)

Taken together, the results obtained here indicated the antiulcer potential of *K. africana*, which validates its popular use in the treatment of gastrointestinal ailments. Importantly, the protective effect of *K. africana* crude extract was observed one hour after the injection of ethanol, indicating that this plant can have protective and curative effects against ethanol-induced ulcer. Furthermore, part of the antiulcer effect of *K. africana* can be attributed to the presence of antioxidant flavonoids and more studies are need to determine which other components of the aqueous plant extract could be also involved in *K. africana* gastroprotective effects.

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